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ORIGINAL ARTICLE

Historical range contraction, and not taxonomy, explains the contemporary genetic structure of the Australian tree *Acacia dealbata* Link

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Abstract

Irrespective of its causes, strong population genetic structure indicates a lack of gene flow. Understanding the processes that underlie such structure, and the spatial patterns it causes, is valuable for conservation efforts such as restoration. On the other hand, when a species is invasive outside its native range, such information can aid management in the non-native range. Here we explored the genetic characteristics of the Australian tree *Acacia dealbata* in its native range. Two subspecies of *A. dealbata* have previously been described based on morphology and environmental requirements, but recent phylogeographic data raised questions regarding the validity of this taxonomic subdivision. The species has been widely planted within and outside its native Australian range and is also a highly successful invasive species in many parts of the world. We employed microsatellite markers to investigate the population genetic diversity and structure among 42 *A. dealbata* populations from across the species' native range. We also tested whether environmental variables purportedly relevant for the putative separation of subspecies are linked with population genetic differentiation. We found no relationship between population genetic structure of *A. dealbata* in Australia and these environmental features. Rather, we identified two geographically distinct genetic clusters that corresponded with populations in the northeastern part of mainland Australia, and the southern mainland and Tasmanian range of the species. Our results do not support the taxonomic subdivision of the species into two distinct subspecies based on environmental features. We therefore assume that the observed morphological differences between the putative subspecies are plastic phenotypic responses. This study provides population genetic information that will be useful for the conservation of the species within Australia as well as to better understand the invasion dynamics of *A. dealbata*.

Keywords Biological invasions · Fabaceae · Genetic diversity and structure · Microsatellites · Tree invasions

Introduction

Range-wide population genetic structure of a taxon is shaped by both biogeographical and ecological factors that impact gene flow among populations. These factors include past glacial and interglacial periods, physical barriers (e.g., mountains, river systems, or oceans), dispersal syndromes, breeding systems, and dispersal of gametes (e.g., pollen flow) (Levin and Kerster 1974; Loveless and Hamrick 1984; Leys et al. 2014; Eidesen et al. 2013). Lack of gene flow is generally considered a prerequisite for the initiation of incipient speciation and, therefore, strong genetic structure is frequently linked to morphological diversification, such as that often observed for subspecies (e.g. *Eucalyptus camaldulensis* in Australia, Butcher et al. 2009).

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Knowledge of population genetic diversity and structure across the native range of a species is crucial for guiding conservation efforts and, when such species are invasive outside their native ranges, to better understand the dynamics underlying invasive populations (Manel et al. 2005; Le Roux et al. 2013; Thomas et al. 2014). Distinct and strong phylogeographic structure in the native range of a species may indicate historical isolation and/or locally adapted lineages. Such information is useful for conservation efforts such as identifying appropriate germplasm sources for local restoration plantings (Dyer and Rice 1997; Cavers et al. 2013; Mijangos et al. 2015). For example, the transfer of genetic material between distinct genetic lineages may change the natural network of genetic connectivity between populations and lead to the breakdown of historical genetic borders and possible loss of evolutionary significance (Crandall et al. 2000; Hufford and Mazer 2003; Jørgensen et al. 2016). Moreover, the mixing of historically isolated gene pools may lead to the dilution of locally adapted genotypes and/or outbreeding depression (Hufford and Mazer 2003). The identification of genetic structures is thus crucial in the context of restoration in order to prevent genetic swamping of unique locally adapted genotypes and to prevent the introduction of genotypes that are potentially maladapted to local environmental conditions (Hufford and Mazer 2003; Jørgensen et al. 2016). Knowledge of genetic structure in native ranges may also be informative where the species has been introduced to new biogeographic regions where it has become invasive. Such information is useful for reconstructing the history of species introductions, in guiding exploration for potential co-evolved biological control agents, and for predicting the spread and potential extent of invasions (Prentis et al. 2009; Thompson et al. 2011; Le Roux et al. 2013; Pyšek et al. 2013).

Australian wattles (genus *Acacia*, formerly grouped in *Acacia* subgenus Phyllodineae, family Fabaceae) are of particular interest for exploring intraspecific genetic diversity and structure in more detail as several species have been used in reforestation and restoration within their native ranges (Murray et al. 2001; Broadhurst et al. 2006). More than a third of the approximately 1063 Australian *Acacia* species (Maslin 2015) are also known to have been moved by humans to areas outside their native ranges (Richardson et al. 2011), and at least 23 species have become invasive (Rejmánek and Richardson 2013). *Acacia dealbata* Link is a particularly interesting species for exploring issues pertaining to natal range population genetic structure. The species is native to southeastern Australia (i.e., New South Wales, Victoria, and eastern Tasmania), where it occurs on tablelands, slopes, and forests and where it has been widely used in restoring land affected by fires and other disturbances (Broadhurst and Young 2006; Poynton 2009; Lorenzo et al. 2010; Peel 2010; Tasmanian Landcare Group 2013). Although *A. dealbata* is a common species in its native range, previous work based on reproductive and genetic characteristics has shown that long-term population persistence, especially for small populations, has been negatively affected by fragmentation (Broadhurst and Young 2006; Broadhurst et al. 2008). The species has also been introduced outside Australia to many regions of the world for multiple purposes (e.g., forestry, horticulture, tannin extraction, perfume production), and is now a widespread invasive species in several countries, notably Chile, Portugal, South Africa, and Spain (Poynton 2009; Lorenzo et al. 2010; Richardson et al. 2011). The genetic diversity and structure of native *A. dealbata* populations is largely unexplored, except for a phylogeographic study by Hirsch et al. (2017). Further research could help to improve conservation approaches within its native range and may provide valuable information in areas where the species is invasive.

Clear genetic structure is expected for *A. dealbata* in Australia, as the taxon comprises two putative subspecies (*A. dealbata* ssp. *dealbata* and *A. dealbata* ssp. *subalpina*) that differ to some extent in their morphology and environmental niches (Kodela and Tindale 2001). Altitude and annual precipitation seem to be the main environmental characteristics separating the two putative subspecies. Occurrences of *A. dealbata* ssp. *dealbata* have been recorded at altitudes up to 1000 m a.s.l. and in areas with annual rainfall of 500–1600 mm, while *A. dealbata* ssp. *subalpina*, which only occurs on the Australian mainland (and not Tasmania), grows mainly above 700 m. a.s.l. with annual rainfall of between 600 and 700 mm (with a few exceptions at lower altitudes) (Kodela and Tindale 2001; www.florabank.org.au, accessed 20 February 2016). *Acacia dealbata* ssp. *dealbata* has also been reported to be taller and have longer leaves than *A. dealbata* ssp. *subalpina* (Kodela and Tindale 2001; www.florabank.org.au). In a previous study, however, we could not differentiate the two putative subspecies using ecological niche modeling approaches and DNA sequencing data from the external transcribed spacer (ETS) gene region (Hirsch et al. 2017). Nevertheless, it is possible that the bioclimatic modeling approach that was applied was not sensitive enough to detect the taxonomic subdivision of *A. dealbata* due to the considerable overlap between the two subspecies ranges (Fig. 1). Alternatively, diversification between the putative subspecies may be recent and therefore not evident in the genetic structure obtained by conservative DNA regions such as that used by Hirsch et al. (2017). Hirsch et al. (2017) emphasized the need for high-resolution population-level genetic analyses to elucidate possible fine-scale population genetic structure and/or putative subspecies.

In this paper, we report on a fine-scale population genetic analysis throughout the native range of *A. dealbata*. Hyper variable microsatellite markers were used to test whether environmental features purportedly relevant for the separation of putative subspecies are linked with population genetic differentiation. To support the hypothesis of separate subspecies,

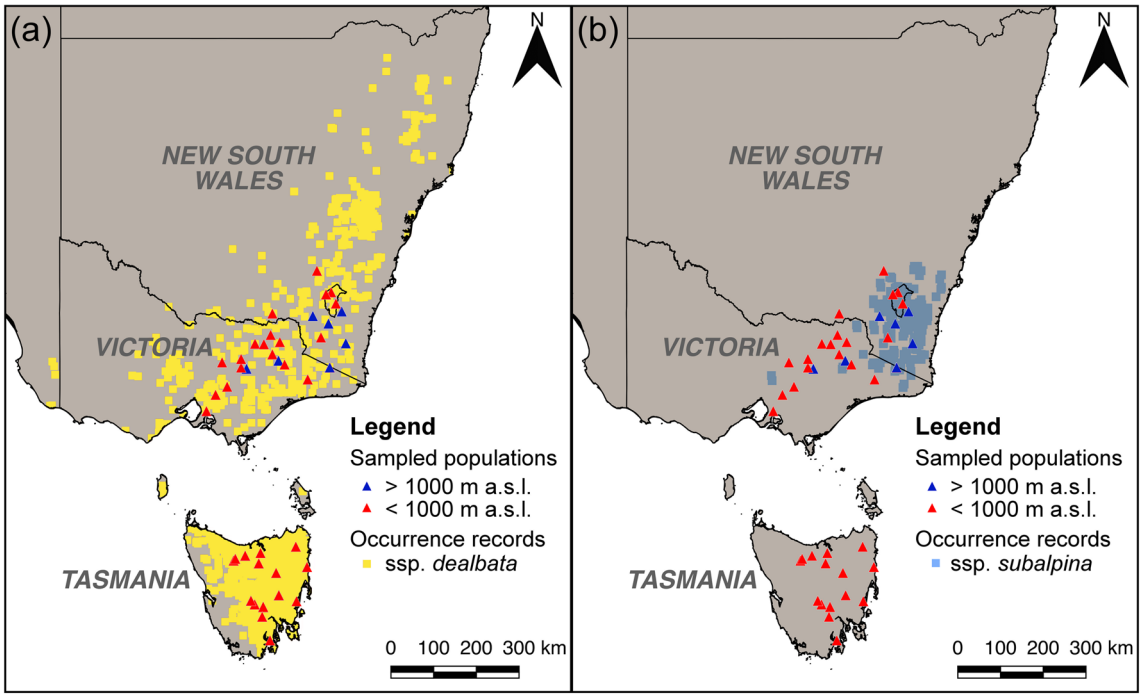

Fig. 1 Native populations of *Acacia dealbata* in Australia sampled for this study (triangles) and the occurrence records of the two putative *A. dealbata* subspecies which were used for the niche modeling approach by Hirsch et al. (2017). The information is shown in two maps for the sake of clarity due to the strong overlap between occurrence records for the putative subspecies. **a** Sampled populations and *A. dealbata* ssp. *dealbata* occurrence records (yellow squares). **b** Sampled populations and *A. dealbata* ssp. *subalpina* occurrence records (blue squares). In both maps, the sample locations are differentiated according to their altitude (i.e., red triangles = location below 1000 m a.s.l.; dark blue triangles = locations above 1000 m a.s.l.)

we predict that population genetic differentiation will be correlated with environmental differentiation (i.e., isolation by environment), but not necessarily with geographic distance (i.e., isolation by distance). The results of this study will help to clarify the taxonomic uncertainty within *A. dealbata.* Such information will also be useful for conservation and restoration efforts in Australia and may be informative on the biogeographic origin and dynamics (e.g., propagule pressure) of invasive *A. dealbata* populations.

Materials and methods

Sampling and DNA extraction

Acacia dealbata leaf material was collected from 42 populations across its native range in the southeastern Australian mainland (27 populations) and in Tasmania (15 populations) (Fig. 1; Table 1). These collections included the holotype location and one of the additional reference locations specified in the description of ssp. *subalpina* by Kodela and Tindale (2001; Table 1) on the mainland. Five other sampled mainland locations occurred at altitudes above 1000 m a.s.l. (Table 1). Based on the information that ssp. *dealbata* occurs generally below 1000 m a.s.l. (Kodela and Tindale 2001; www.florabank.org.au, accessed 20 February 2016), we assumed that these sites represent additional populations of the putative ssp. *subalpina*.

For each population, fresh leaves that showed no signs of disease or mold were sampled from 20 randomly chosen individuals (total $n = 840$). Collected leaves were stored on silica gel until further use.

DNA extraction from dried leaf tissue was performed following the cetyltrimethylammonium bromide (CTAB) protocol (Doyle and Doyle 1990) with the modification of adding 0.2 M sodium sulfite to the extraction buffer for minimization of DNA degradation (Bryne et al. 2001). All DNA extractions were diluted to a standard concentration of 100 ng μl^{-1}.

Genotyping

Twenty nuclear microsatellite loci previously characterized for *A. dealbata* (Guillemaud et al. 2015) were initially tested for amplification success and polymorphism in a subset of samples. Ten of these loci showed consistent and good-quality amplification products and were therefore used for further genotyping of all *A. dealbata* samples (Table S1, Online Resource). Microsatellite amplification was performed in two multiplex PCR assays for which primers with non-overlapping allele-size ranges and/or different 5′-dyes were combined (Table S1, Online Resource). All PCR reactions were carried out in a volume of 10 μl containing 2 μl template

Table 1 Locations and genetic characteristics of the studied native populations of *Acacia dealbata* in the southeastern Australian mainland and Tasmania. For each population, the coordinates in decimal degree (Lat = latitude; Long = longitude), the altitude in meters above sea level (Alt), the number of samples (N), allelic richness (A_R), observed (H_O) and expected (H_E) heterozygosity, and inbreeding coefficients (F_{IS}) are provided. Mean ancestry coefficients (Q) are shown for cluster 1 (Q1) and cluster 2 (Q2) according to the STRUCTURE results for $K = 2$ (Fig. 2; Fig. S2, Online Material). Populations (PopID) marked with an asterisk occurred above 1000-m elevation and were assumed to represent putative *A. dealbata* ssp. *subalpina* according to the altitude criteria described in Kodela and Tindale (2001). These sampled populations included the holotype location (AUS_22) and another reference location (AUS_19) used by Kodela and Tindale (2001) for the description of ssp. *subalpina*

PopID	Lat	Long	Alt	N	A_R	H_O	H_E	F_{IS}	Q1	Q2
Tasmania										
TAS_1	−41.51	146.08	746	19	3.79	0.46	0.46	−0.010	0.09	0.91
TAS_2	−41.47	146.13	157	18	3.98	0.44	0.45	0.052	0.08	0.92
TAS_3	−41.40	146.42	154	18	3.66	0.49	0.46	−0.074	0.07	0.93
TAS_4	−41.57	146.82	212	18	3.67	0.40	0.47	0.137	0.16	0.84
TAS_5	−41.78	147.33	201	14	3.55	0.47	0.49	0.056	0.28	0.72
TAS_6	−42.27	147.41	425	20	2.94	0.60	0.51	−0.211	0.27	0.73
TAS_7	−42.52	146.95	373	19	1.69	0.47	0.27	−0.556	0.07	0.93
TAS_8	−42.47	146.70	132	17	3.04	0.49	0.42	−0.115	0.09	0.91
TAS_9	−42.39	146.59	325	17	4.24	0.51	0.53	0.031	0.09	0.91
TAS_10	−42.73	146.92	32	19	3.42	0.51	0.42	−0.156	0.11	0.89
TAS_11	−43.24	147.15	20	19	3.80	0.49	0.53	0.054	0.05	0.95
TAS_12	−42.40	147.93	81	20	2.55	0.42	0.34	−0.181	0.61	0.39
TAS_13	−41.65	148.24	312	17	3.96	0.53	0.52	−0.002	0.12	0.88
TAS_14	−41.20	147.91	362	17	4.18	0.52	0.56	0.079	0.06	0.94
TAS_15	−41.34	146.87	117	20	3.57	0.47	0.49	0.037	0.15	0.85
Mean					3.47	0.49	0.46			
Standard deviation					0.68	0.05	0.08			
Australian mainland										
AUS_1	−38.13	145.28	71	18	3.61	0.50	0.51	0.039	0.21	0.79
AUS_2	−37.75	145.55	126	20	3.76	0.53	0.51	−0.051	0.39	0.61
AUS_3	−37.56	145.89	863	19	4.53	0.56	0.57	0.015	0.22	0.78
AUS_4	−36.99	145.74	326	19	2.41	0.49	0.34	−0.362	0.93	0.07
AUS_5*	−37.14	146.46	1390	18	4.65	0.53	0.56	0.095	0.38	0.62
AUS_6	−37.11	146.30	531	19	2.57	0.50	0.44	−0.130	0.91	0.09
AUS_7	−36.91	146.30	852	19	3.33	0.53	0.47	−0.124	0.64	0.36
AUS_8	−36.55	146.71	221	18	4.23	0.55	0.59	0.055	0.64	0.36
AUS_9	−36.34	147.17	221	18	3.32	0.42	0.43	0.008	0.82	0.18
AUS_10	−36.51	147.44	311	19	3.34	0.49	0.45	−0.014	0.87	0.13
AUS_11	−36.56	146.97	577	18	3.85	0.53	0.52	−0.012	0.86	0.14
AUS_12	−36.80	147.22	571	17	2.97	0.52	0.41	−0.194	0.81	0.19
AUS_13*	−36.95	147.40	1360	18	3.16	0.53	0.43	−0.185	0.85	0.15
AUS_14	−37.04	147.58	679	19	3.28	0.49	0.47	0.001	0.79	0.21
AUS_15	−37.39	148.26	672	17	4.13	0.50	0.51	0.012	0.85	0.15
AUS_16*	−35.89	148.41	1411	19	2.00	0.60	0.37	−0.581	0.92	0.08
AUS_17*	−36.07	148.87	1210	17	1.98	0.40	0.28	−0.214	0.24	0.76
AUS_18	−36.40	148.65	965	18	3.64	0.45	0.46	0.029	0.70	0.30
AUS_19*	−37.11	148.90	1311	20	2.10	0.48	0.40	−0.202	0.96	0.04
AUS_20	−37.11	148.91	929	17	2.72	0.29	0.32	0.128	0.90	0.10
AUS_21*	−36.54	149.38	1136	19	3.54	0.48	0.51	0.000	0.28	0.72
AUS_22*	−35.78	149.26	1114	18	3.39	0.59	0.52	−0.109	0.79	0.21
AUS_23	−35.59	149.09	750	19	3.01	0.56	0.47	−0.098	0.71	0.29
AUS_24	−35.32	148.95	579	18	2.85	0.56	0.46	−0.224	0.91	0.09
AUS_25	−35.37	148.80	1167	18	3.71	0.44	0.48	0.067	0.47	0.53
AUS_26	−34.80	148.53	527	17	3.75	0.47	0.52	0.095	0.71	0.29
AUS_27	−35.83	147.22	347	17	2.87	0.49	0.44	−0.111	0.75	0.25
Mean					3.28	0.50	0.46			
Standard deviation					0.71	0.06	0.07			

DNA (100 ng/μl), 5 μl KAPA2G Fast Multiplex Mix (Kapa Biosystems, Cape Town, South Africa), 1 μl primer mix (concentration of each primer provided in Table S1, Online Resource) of the corresponding multiplex set (Thermo Scientific, Waltham, Massachusetts, USA), and 2 μl purified H_2O. PCR cycling for both multiplexes was performed in a MultiGene OptiMax thermal cycler (Labnet International, Edison, New Jersey, USA) with an initial denaturation of 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, elongation at 72 °C for 30 s, and a final elongation at 72 °C for 10 min. Each 96-well PCR plate contained 92 samples plus three

randomly selected replicate samples and one negative control (H_2O). Amplification products were submitted for gel capillary electrophoretic separation at the Central Analytical Facility, Stellenbosch University, Stellenbosch, South Africa. GeneMarker software (version 2.6.4; SoftGenetics LLC, Pennsylvania, USA) was used for genotype scoring by using marker panels to call the alleles which were then manually checked. Out of 840 samples, reliable and high-quality genotypes were obtained for 765 samples (Table 1).

Genetic diversity

The full data set was checked for the presence of null alleles and scoring errors with Micro-Checker version 2.2 (Van Oosterhout et al. 2004). In this approach, null alleles were detected and their frequencies estimated at each locus and population according to the expected maximization approach as implemented in the software FreeNA (Chapuis and Estoup 2007). FreeNA was also employed to calculate uncorrected and corrected (i.e., excluding null alleles; so-called ENA method as described in Chapuis and Estoup 2007) estimates of pairwise F_{ST} values following Weir (1996). All loci were tested for allele frequency departures from Hardy-Weinberg equilibrium (HWE) expectations using the packages adegenet version 2.0.1 (Jombart 2008) and pegas version 0.9 (Paradis 2010) in R (R Core Team 2016).

Allelic richness (A_R) and observed and expected heterozygosity (H_O and H_E) were calculated as measures of diversity per population with the package diveRsity (Keenan et al. 2013). Rarefaction, as implemented in the diveRsity package, was applied for the A_R calculations to account for the unbalanced sample sizes among populations. The package diveRsity was used to calculate inbreeding coefficients (F_{IS}) per population.

Genetic structure

A combination of Bayesian clustering, multivariate ordination, and dissimilarity matrix correlations was applied to identify the genetic structure among *A. dealbata* populations. First, the program STRUCTURE version 2.3.4 (Pritchard et al. 2000) was used to detect the number of genetic clusters (K) present in our dataset. We tested values of K varying from 1 up to 23 and employed an admixture model with correlated allele frequencies, 100,000 burn-in iterations, 500,000 Markov Chain Monte Carlo repetitions, and 20 iterations per run. The optimum number of K was obtained following the delta K method of Evanno et al. (2005) using the online software STRUCTURE HARVESTER (version 0.6.94; Earl and vonHoldt 2012). The programs CLUMPP (version 1.1.2; Jakobsson and Rosenberg 2007) and DISTRUCT (version 1.1; Rosenberg 2004) were used for graphical visualization of the STRUCTURE results. To graphically explore whether the optimum number of K translates into geographical patterns, we applied a spatial interpolation of the ancestry coefficients found for the optimal K clusters by using the *maps* function implemented in the POPSutilities.R suite R package (Jay et al. 2012). For illustrative purposes, we also incorporated the sequencing results from Hirsch et al. (2017) for all populations included in the current study by replotting the parsimony network and depicting the spatial distribution of ETS-based haplotypes. The parsimony network was reconstructed following Hirsch et al. (2017) using the R package pegas (version 0.10; Paradis 2010).

A principal coordinate analysis (PCoA) was used to visualize the genetic relationships between the native *A. dealbata* populations. The PCoA was conducted with the package vegan version 2.4-2 (Oksanen et al. 2017) using the uncorrected genetic distances (Cavalli-Sforza and Edwards 1967) calculated with FreeNA. A between-class analysis was performed as implemented in the package ade4 version 1.7-6 (Dray and Dufour 2007) by predefining groups of populations according to the genetic clusters found with STRUCTURE and the spatial interpolation.

Because STRUCTURE identified two genetic clusters, while the PCoA results did not show a clear distinction between two genetic clusters (see "Results"), we investigated the pairwise F_{ST} values in more detail to test whether the STRUCTURE approach might have resulted in false estimation of genetic structure, i.e., the existence of one genetic cluster. To do this, we calculated pairwise F_{ST} values of populations that were characterized by ancestry coefficients (Q-values) ≥ 0.9 to one of the two genetic clusters (Table 1; Fig. 2; Table S2, Online Resource) and then tested whether genetic differentiation is higher between compared to within these two clusters. For doing so, pairwise F_{ST} values were logit transformed (Warton and Hui 2011) and Welch's t test (i.e., unequal variance t test) was performed in R (R Core Team 2016).

We performed a combination of Mantel tests, partial Mantel tests, and multiple matrix regression analyses with randomization (MMRR; Wang 2013) to test for genetic isolation by distance (IBD) and/or isolation by environment (IBE) among native *A. dealbata* populations in Australia. MMRR is regarded as a robust statistical tool to estimate independent effects of potential factors (Wang 2013). Linearized uncorrected pairwise F_{ST} values (i.e., $F_{ST}/1-F_{ST}$) were used as genetic distances between populations. Geographic distances between populations were calculated from their GPS coordinates with the Geographic Distance Matrix Generator version 1.2.3 (Erst 2017). The bioclimatic variables (temperature seasonality, maximum temperature of the warmest month, minimum temperature of the coldest month, and annual precipitation) were used in the *A. dealbata* niche modeling study by Hirsch et al. (2017). We also used altitude, as this was previously described as one of the main proxies for subspecies classification (Kodela and Tindale 2001). For each population,

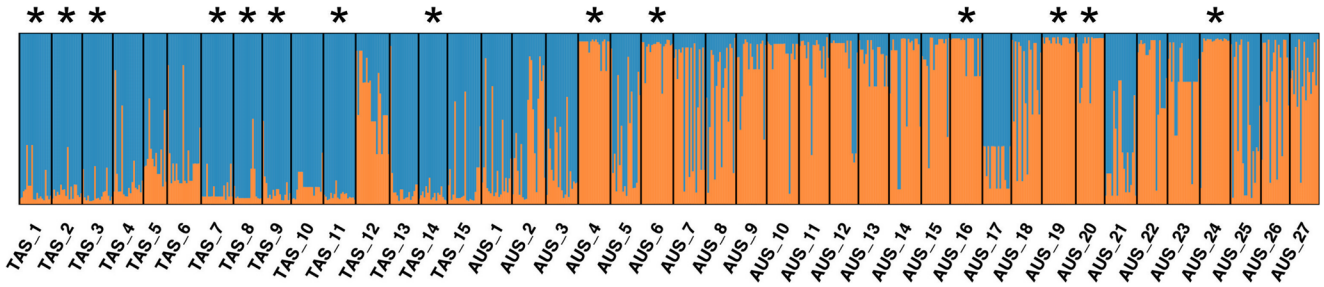

Fig. 2 STRUCTURE results for the native populations of *Acacia dealbata*. The analysis revealed two genetic clusters ($K = 2$) as optimal number and populations which were assigned to one of the two clusters by an average ancestry coefficient (Q) higher or equal to 0.9 are indicated by asterisks above the bar plot. The names underneath the bar plot refer to the population IDs shown in Table 1

environmental variables were downloaded from the WorldClim database (Hijmans et al. 2005) at a resolution of ~ 5 km. A principal component analysis was performed on the five environmental variables using the R package vegan version 2.4-2 (Oksanen et al. 2017). The scores of the first two principal components were used to calculate the environmental distances as squared Mahalanobis distances between populations (Mahalanobis 1936). However, as altitude and annual precipitation are thought to be the main environmental parameters driving differentiation the two *A. dealbata* subspecies (Kodela and Tindale 2001), it is conceivable that their effects on the genetic differentiation may be obscured when pooled as Mahalanobis distances. For this reason, we also calculated additional environmental distance matrices that only contained the corresponding Euclidean distances for altitude and annual precipitation separately using the R package vegan version 2.4-2 (Oksanen et al. 2017). Mantel tests to test for IBD and IBE were carried out with the corresponding distance matrices (geographic and environmental respectively, and genetic) containing all sampled populations and with matrices containing subsets of populations according to the genetic clusters identified by STRUCTURE and the spatial interpolation approach (see "Results"). A similar procedure was used for the partial Mantel test but tests for IBD were controlled for by the corresponding environmental distance matrix (i.e., Mahalanobis distance), while tests for IBE were controlled for by the corresponding geographical distance matrix. The MMRR models were conducted for the complete dataset and for the genetic clusters separately. In each case, one model was formulated with the geographic and environmental (i.e., Mahalanobis) distances as independent variables and a second model with geographic distance, environmental distance based on altitude, and environmental distance based on annual precipitation as independent variables. Genetic distance was always used as the dependent variable. Before running the MMRR models, all distance matrices were standardized by subtracting the mean and dividing by the standard deviation. All Mantel tests and MMRR models were performed with 999 permutations. The vegan R package version 2.4-2 (Oksanen et al. 2017) was used for the Mantel tests and the MMRR function script (Wang 2013) was used for the MMRR models. In cases of significance, the relationships between the two corresponding distances were depicted graphically in scatterplots and with a two-dimensional kernel density estimation as implemented in the R package MASS version 7.3-45 (Venables and Ripley 2002). This approach allowed us to identify whether the significant correlation is attributable to continuous clines of genetic differentiation or to disjunct patches of populations (Jombart 2015).

To assess the genetic variation between and among the genetic clusters identified by our STRUCTURE analysis and the spatial interpolation approach, a hierarchical analysis of molecular variance (AMOVA) was performed using the R package poppr version 2.3.0 (Kamvar et al. 2014 2015).

Results

Genetic diversity

No scoring errors associated with band stuttering were identified. All ten loci were polymorphic and between five and 17 alleles were detected per locus (Table S1, Online Resource). However, nine loci showed significant departures from HWE for at least three sampled populations (Table S1, Online Resource). Also, 123 out of 420 locus-by-population comparisons did not meet the expectations under HWE (Fig. S1, Online Resource). A low average null allele frequency was detected (0.025, s.d. = 0.054). When correcting for the presence of these, no significant difference was detected between the corrected and uncorrected pairwise F_{ST} values (Kruskal-Wallis chi square = 0.345, $P = 0.557$). We therefore decided to conduct all subsequent analyses without correction for null alleles.

A_R values per population ranged between 1.69 and 4.65, while H_O and H_E ranged from 0.29 to 0.60 and 0.27 to 0.59, respectively (Table 1). On average, Australian mainland and Tasmanian populations were characterized by similar diversity measures, and all populations showed no or only very low levels of inbreeding (Table 1).

Genetic structure

Two genetic clusters ($K = 2$) were identified by STRUCTURE as the optimal number among the native *A. dealbata* populations (Fig. 2; Fig. S2, Online Resource). Spatial interpolation showed that cluster 1 predominantly comprised northeastern part of the native range while cluster 2 corresponded mostly to Tasmanian and the southern mainland range (Fig. 3). When compared with the spatial distribution of the haplotypes identified for the same populations by Hirsch et al. (2017), this differentiation was also reflected, to some extent, among ETS haplotypes (Fig. 3). This structure becomes most apparent in the distribution of one of the most frequently detected haplotypes (haplotype I), which was represented in nine out of the 18 populations of cluster 2 but in none in cluster 1 (Fig. 3). Apart from two widespread haplotypes (II and III), two private haplotypes (IV and V) corresponded to cluster 2 while four (VI, VII, VIII, and X) corresponded to cluster 1 (Fig. 3).

Visualization by PCoA supported the two genetic clusters identified in STRUCTURE to some extent, indicating a separation between most Australian mainland and Tasmanian populations, but rather along a gradient than by distinct clusters (Fig. S3, Online Resource). However, the regional differentiation was more clearly supported by the between-class analysis which showed only very little overlap between both classes (i.e., cluster 1 vs. cluster 2) (Fig. S4, Online Resource). The genetic differentiation between the two identified clusters was significantly higher than within clusters (Welch *t* test: $t = 5.62$, $P < 0.01$; Fig. S5, Online Resource), which supports the STRUCTURE results of an optimal $K = 2$. Higher levels of genetic clustering by STRUCTURE (i.e., $K > 2$) supported the distinctiveness of the two clusters identified as optimal (data not shown). Nevertheless, it should be noted that some of the populations within the two geographical regions showed clear admixture between cluster 1 and cluster 2 (e.g., populations TAS_12, AUS_17, AUS_21; Fig. 2; Fig. S3, Online

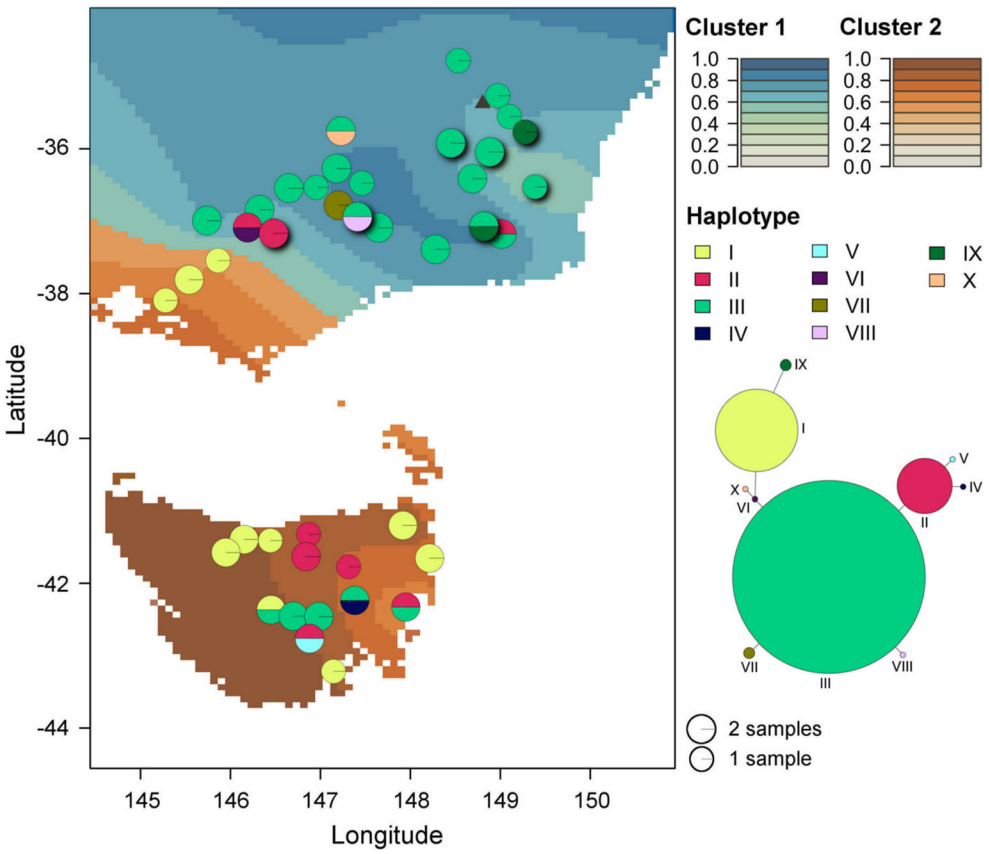

Fig. 3 Spatial interpolation of the ancestry coefficients estimated by STRUCTURE for the optimal number of genetic clusters ($K = 2$) among native population of *Acacia dealbata* in southeastern Australia. Ancestry coefficients with maximal local contribution to ancestry are shown in blue for cluster 1 and in orange for cluster 2. The higher the corresponding ancestry coefficient, the darker the color (see legend). Locations of the *A. dealbata* populations studied in this study are represented by pie charts which indicate the identity of ETS haplotypes identified using the sequencing data from Hirsch et al. (2017). For one population, indicated by a black triangle, no sequencing data were available. The seven pie charts on the Australian mainland which cast a shadow symbolize the populations with putative ssp. *subalpina* (i.e., locations > 1000 m a.s.l.; Table 1). The parsimony network based on the ETS sequences is shown in the legend to illustrate the relationships between the corresponding haplotypes. Short connection lines between two haplotypes in the network translate into one mutation step while longer connections lines (i.e., between haplotype I and VI, and between I and IX) translate into three mutation steps

Resource). In regard to the subspecies context, the PCoA did not show any clear differentiations between the putative ssp. *subalpina* and ssp. *dealbata* (Fig. S3, Online Resource).

The Mantel tests and MMRR models revealed highly significant IBD across all sampled populations of *A. dealbata*, caused by two distant patches of populations (Table 2; Fig. 4). When the sub-datasets for the genetic clusters were tested separately, a marginal significant IBD was found for cluster 1 (mostly Australian mainland populations) (Table 2), caused by a continuous cline of genetic differentiation (Fig. S6, Online Resource). However, the IBD for cluster 1 was only marginal or not significant when applying MMRR, while none of the approaches found evidence for IBD within cluster 2 (Table 2). All tests for IBE were non-significant, indicating that environmental factors included here are not linked to population genetic diversification of *A. dealbata* (Table 2). One exception was a significant signal when genetic distance was tested with the environmental distance based on only altitude using a Mantel test for the complete dataset (Table 2). However, after correcting this relationship with geographic distance in the partial Mantel test or using the MMRR approach, no significant effect by altitude could be detected (Table 2).

The hierarchical AMOVA results indicated low (5.4%) but significant ($P = 0.01$) differentiation of genetic variance between the two genetic clusters of native *A. dealbata* populations and considerable genetic variation among populations (22.7%), while most variation resided within populations (71.9%) (Table 3).

Discussion

We found no evidence that differentiation between *Acacia dealbata* populations in Australia was linked to environmental features, including those supposedly linked to the differentiation of the two subspecies. The lack of significant IBE and the corresponding genetic structure supports the findings of our previous work which proposed that the reported morphological differences among native *A. dealbata* populations (Kodela and Tindale 2001) might be mediated by high phenotypic plasticity rather than genetically based differences between two subspecies (Hirsch et al. 2017). Rather, the genetic structure across the native range of *A. dealbata* identified here seems to be linked to geographical features. One of the most obvious geographic features that could explain this pattern is the Bass Strait, which separates Tasmania from mainland Australia. Genetic divergences across the Bass Strait have been identified in numerous other native Australian plant taxa with contemporary distributions similar to *A. dealbata* (e.g., *Atherosperma moschatum*, Worth et al. 2011; *Eucalyptus regnans*, Neville et al. 2010; *Nothofagus cunninghamii*, Worth et al. 2009, Duncan et al. 2016; *Tasmannia*

Table 2 Results of the Mantel tests, partial Mantel tests, and multiple matrix regressions with randomization (MMRR) performed among all investigated native populations of *Acacia dealbata* and among the groups of populations according to the STRUCTURE and spatial interpolation results (cluster 1 populations: Australian mainland populations excluding AUS_1, AUS_2, and AUS_3; cluster 2 populations: Tasmanian populations plus the Australian mainland populations AUS_1, AUS_2, and AUS_3). *IBD*, isolation by distance; *IBE*, isolation by environment; *GEN*, genetic distance (i.e., pairwise F_{ST} values); *GEO*, geographic distance; *ENV*, Mahalanobis distances based on all considered environmental variables; ENV_{alt}, environmental distance based only on altitude; ENV_{Bio12}, environmental distance based only on annual precipitation. For the IBD relations, MMRR results with no parentheses refer to the values revealed from the regression of GEN with ENV and GEO while values in parentheses refer to values from the regression of GEN with ENV_{alt}, ENV_{Bio12}, and GEO. For the Mantel tests and partial Mantel tests, the Mantel statistic (r) and the significance level (P) are provided. For the MMRR models, the corresponding correlation coefficients (β) and P values are provided. Italicized numbers highlight significant results

Region	Tested relation	Mantel test		Partial Mantel test		MMRR	
		r	P	r	P	β	P
Complete	IBD: GEN × GEO	0.208	*< 0.01*	0.218	*< 0.01*	0.249 (0.354)	*< 0.01 (< 0.01)*
	IBE: GEN × ENV	0.036	0.31	− 0.076	0.76	−0.084	0.44
	IBE: GEN × ENV_{alt}	0.116	*0.02*	− 0.095	0.88	−0.186	0.17
	IBE: GEN × ENV_{Bio12}	0.030	0.34	0.017	0.38	0.046	0.65
Cluster 1	IBD: GEN × GEO	0.173	*0.04*	0.177	*0.03*	0.181 (0.178)	0.05 (0.07)
	IBE: GEN × ENV	− 0.036	0.56	− 0.052	0.60	−0.050	0.72
	IBE: GEN × ENV_{alt}	0.032	0.36	0.016	0.43	0.024	0.79
	IBE: GEN × ENV_{Bio12}	− 0.018	0.48	− 0.035	0.53	−0.039	0.78
Cluster 2	IBD: GEN × GEO	0.018	0.31	0.028	0.31	0.029 (0.043)	0.90 (0.83)
	IBE: GEN × ENV	− 0.051	0.47	− 0.056	0.50	−0.055	0.72
	IBE: GEN × ENV_{alt}	− 0.089	0.64	− 0.098	0.71	−0.204	0.16
	IBE: GEN × ENV_{Bio12}	0.043	0.28	0.041	0.29	0.165	0.27

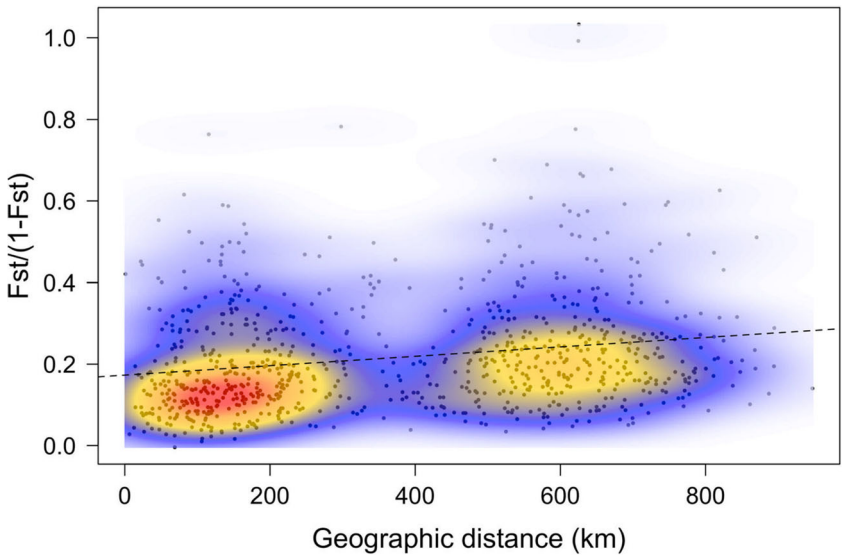

Fig. 4 Correlation between genetic and geographic distances among all investigated native populations of *Acacia dealbata*. A two-dimensional kernel density estimation was added to the graph. Higher point densities are indicated by warmer colors and lower densities by colder colors. All geographic distances above 471 km represent distances between Australian mainland and Tasmanian populations. Distances below 471 km indicate mainly pairs of populations within the two geographical regions

lanceolata, Worth et al. 2010; see also Byrne et al. 2011). Such information is helpful for identifying regions of long-term survival (i.e., refugia) and spatial and temporal fluctuations of taxon ranges in the past. In the case of *A. dealbata*, the distribution of all three high-frequency ETS genotypes (i.e., I, II, and III, Fig. 3) on both sides of the Bass Strait might indicate that historical land bridges across the strait facilitated the long-term survival of a nearly continuous population which was separated due to rising sea levels at the end of the Last Glacial Maximum (25,000–15,000 year B.P., Chapple et al. 2005; Lambeck et al. 2014). On the other hand, it could also be argued that the spatial interpolation of the STRUCTURE results, as well as the distribution of haplotype I (Fig. 3), points to the possibility that the main boundary between the clusters is situated on the mainland (e.g., south-western spurs of the Australian Alps). Further research is needed to elucidate and substantiate details on the historical range fluctuations of *A. dealbata*. Approaches considering fossil pollen records, sequencing of chloroplast DNA regions, and paleobiogeographic niche modeling would be helpful in this regard (e.g., Schaal et al. 1998; Alba-Sánchez et al. 2010; Varela et al. 2011; Worth et al. 2014).

In terms of genetic diversity and structure in *A. dealbata*, our results provide information that will be useful for guiding conservation and restoration efforts in Australia. For example, we found evidence for the occurrence of gene flow between the two main genetic clusters identified here. Such gene flow seems to be most likely between mainland populations in the contact zone of both genetic clusters, supported by the high admixture (i.e., low ancestry coefficients to anyone particular cluster) for these populations (e.g., populations AUS_1, AUS_2, AUS_3; Fig. 3). Nevertheless, the between-class analysis and the significant IBD indicated a general genetic distinctiveness between most of the Australian mainland populations and the Tasmanian populations. This indicates that transfer of genetic material between these two regions should be avoided (e.g., see van der Mijnsbrugge et al. 2010; Jørgensen et al. 2016). However, it is important to note that some populations within both geographical regions showed a high admixture between both genetic clusters. Due to the lack of more detailed information on the individual sampling sites, the causes of such admixture remain unknown. One reason might be the transfer of genetic material between the two clusters due to previous mixing of populations through translocation by humans. Although we avoided sampling at sites which looked like restoration plantings, we cannot exclude the possibility that some populations were not true natural populations.

Future planning of restoration programs within the corresponding regions should, in any event, exercise caution to prevent further admixture between the two genetic clusters, supported by the genetic differentiation between them, several

Table 3 Hierarchical analysis of molecular variance (AMOVA) for all investigated native populations of *Acacia dealbata* under consideration of the two genetic clusters identified with STRUCTURE and the spatial interpolation

Source of variation	df	Sum of squares	Variance	Percent variation (%)
Between clusters	1	126.9	0.273	5.4
Among populations	40	981.7	1.148	22.7
Within populations	723	2632.7	3.640	71.9

private haplotypes within each cluster, and the significant IBD, all of which are indicative of a high potential of spatially sorted genotypes (Pannell and Fields 2014; Sjöstrand et al. 2014). Another important task for restoration efforts is highlighted by the considerable genetic variation among the *A. dealbata* populations; such diversity should be preserved to maintain the total genetic diversity of the species. This aspect should be considered in conservation measures to counteract a genetic depletion in natural *A. dealbata* populations which are negatively affected by fragmentation (Broadhurst and Young 2006; Broadhurst et al. 2008). Besides consulting the genetic information provided in this study to select source and recipient populations for conservation projects, we also recommend that common-garden or reciprocal-transplanting experiments should be done to identify the best source population(s) for restoration projects in different areas (Gibson et al. 2016; Jørgensen et al. 2016). Such approaches are more useful to assess adaptive variation within and among populations (Hufford and Mazer 2003). Moreover, this could also help to test whether the previously described morphological differences between the putative subspecies of *A. dealbata* (Kodela and Tindale 2001) are environmentally driven plasticity (e.g., Byars et al. 2007; Mathiasen and Premoli 2016). Moreover, our results provide baseline data that can be used to infer the route(s), source(s), propagule pressure, etc. underlying *A. dealbata* invasions globally. For example, because records of the history of introduction of species are often incomplete or inaccurate (Le Roux et al. 2011; Hirsch et al. 2011), molecular methods offer the best insights in this regard and can provide information on the genetic characteristics (e.g., genetic bottlenecks) and dynamics (e.g., number of introduction events) of invasive populations (Le Roux and Wieczorek 2009).

Conclusions

Together with our previous findings (Hirsch et al. 2017), the results of this study highlight the need to reassess the current taxonomic subdivision of *A. dealbata* into two subspecies. The genetic structure of native populations of *A. dealbata* may reflect plastic phenotypic responses as responsible for the altitudinal differences observed between the assumed "subspecies." However, we recommend that common-garden and/or reciprocal-transplant experiments be conducted to conclusively test this. Such approaches may also reveal morphological differences to rather reflect locally adapted genotypes, which may not be detected by neutral genetic markers such as those employed in the current study. More research is also needed to elucidate the historical population dynamics within the native range of *A. dealbata* to improve our knowledge of spatio-temporal genetic dynamics and characteristics in the Australian flora which are surprisingly poorly understood in a phylogeographic context (Neville et al. 2010). This is the first study to provide detailed information on population genetics across the large native range of *A. dealbata*. The results are important for guiding management of the species, both in its native range and in other parts of the world where it is an important invader.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Data archiving statement

External transcribed spacer sequencing data used in this study will be available at GenBank. Accession numbers will be included as supplementary material once archiving is completed.

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